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Cortisol and Working Memory In Boys With Fragile X Syndrome

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CORTISOL AND WORKING MEMORY IN BOYS WITH FRAGILE X SYNDROME

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ABSTRACT

Fragile X Syndrome (FXS) is a neurodevelopmental disorder and the most common cause of inherited intellectual disability. Although FXS is associated with global cognitive impairments, specific deficits in working memory have been reported in young males with FXS. Working memory is an important cognitive process that involves the ability to temporarily store and manipulate information over a short period of time. Deficits in working memory can negatively impact an individual's academic, behavioral, and social functioning. Chronic stress can adversely influence working memory performance and can be measured physiologically through salivary cortisol. It is important to study the complex relationship of how physiological and cognitive processes interact and develop over time to aid in the specificity of assessments and treatments for individuals that are vulnerable to develop cognitive impairments over time. The present study investigates the relationship of developmental trajectories of working memory performance in boys with FXS compared to typically developing boys. This study also examined the relationship of salivary cortisol on memory performance over time in boys with FXS and typically developing boys. Results from multilevel models indicate specific cognitive deficits in working memory performance in boys with FXS compared to typically developing boys. No significant differences were seen in working memory trajectories between boys with FXS and typically developing boys after controlling for mental age. Results further indicated that boys with FXS had higher levels of baseline cortisol that negatively impacted working memory performance over time compared to

typically developing boys. This study highlights the need for further investigation on how dynamic physiological and cognitive factors interact and influence an individual's cognitive development over time.

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CHAPTER 1

INTRODUCTION

There has been a concentrated effort to better understand how biological and environmental influences interact and contribute to the development of cognitive functioning over time (Jordan & Wüstenberg, 2010). By understanding multiple, complex, dynamic systems involved in cognitive development, we can better examine the emergence of underlying mechanisms that impact cognition under various contexts (Karmiloff-Smith, 1998). Working memory is an important facet of cognition that impacts many higher-level cognitive processes involved in an individual's academic, behavioral, and social functioning. However to date, few research studies have examined the relationship of biological factors that may impact working memory development over time in both typical and atypical populations, such as FXS.

1.1 **Fragile X Syndrome**

Fragile X syndrome (FXS) is a neurodevelopmental disorder that is the most common cause of inherited intellectual disability (Hagerman, 2008; Crawford et al., 2002) and affects approximately 1 in 4,000 males (Crawford, Acuña, &Sherman, 2001). FXS is a genetic disorder that is caused by changes in the fragile X mental retardation 1 (FMR1) gene. FMR1 produces fragile X mental retardation protein (FMRP), which is a needed and critical component for brain development (Bassell & Warren, 2008; Brown et al., 2001; Eichler et al., 2004). Individuals with FXS have an expansion of CGG repeats

on the FMR1 gene that exceeds 200 copies, and are classified as having the full mutation of the syndrome, while the premutation of fragile X contains 55-200 CGG repeats (Fu et al., 1991; Snow et al., 1993).

Males and females are both affected by FXS; however, females present with a more variable cognitive phenotype. The majority of adult males with FXS are diagnosed with intellectual disabilities in the moderate to severe range (Merenstein et al., 1996), while the majority of females with FXS will have intellectual abilities that fall within the borderline range (70-84) (De Vries et al., 1996) or above. FXS is also highly comorbid with anxiety (Bailey, Raspa, Olmsted, & Holiday, 2008), autism (Bailey, Hatton, Mesibov, Ament, & Skinner, 2000; Hatton et al., 2006), hyperarousal (Roberts, Boccia, Bailey, Hatton, & Skinner, 2001) and ADHD (Sullivan et al., 2006). However, despite these cognitive and behavioral vulnerabilities, little is known about the developmental trajectories of how these deficits develop over time in individuals with FXS.

In addition to a global intellectual impairment, specific cognitive deficits in the areas of visual-spatial processing (Cornish, Munir, & Cross, 1999), sequential processing (Cornish et al., 2004), and executive functioning (Munir, Cornish, & Wilding, 2000) have been documented in FXS. Although a wide array of cognitive deficits are associated with FXS, specific impairments in working memory have been reported as particularly impairing (Baker et al., 2011; Munir, et al., 2000).

1.2 Working Memory

Working memory involves the ability to simultaneously store and manipulate information over a short period of time. Baddeley's (1986) model of working memory is

characterized by three subsystems: the central executive, the phonological loop, and the visual-spatial sketchpad. The central executive subsystem is responsible for processing and manipulating information. The other two subsystems involve domain-specific aspects for processing verbal and visuospatial information. The phonological loop processes information that has verbal or linguistic qualities, while the visual-spatial sketchpad performs mental operations that contain visualspatial information (Baddeley, 2000). One important feature of Baddeley's model of working memory is that these subsystems play an active and integrated role in facilitating working memory.

There is a limited capacity to the amount of information that can be held and processed in an individual's working memory. When increased demands are put on the central executive, such as tasks that require greater processing or manipulation information, less attention and energy will be allocated to the phonological loop and visual-spatial sketchpad subsidiary subsystems. However, working memory capacity increases with maturation during childhood in typically developing children and eventually stabilizes in adulthood (Alloway, Gathercole, & Pickering, 2006; Case, Kurland, & Goldberg, 1982; Towse, Hitch, & Hutton, 1998). Gathercole et al. (2004) examined each component of the Baddeley model (i.e. central executive, visual-spatial sketchpad, and phonological loop) in a sample of typically developing children 4-15 years of age and found positive linear relationships on all the measures of the working memory model as a function of age.

Each of these subsystems play an important role in the storage, retrieval, and processing of information. When any of these subsystems are disrupted, deficits may be seen on specific tasks depending on which subsystem is affected (Henry &Winfield,

2010). For example, an individual with impairment in the phonological loop system of working memory may have difficulty on tasks that involve verbal or linguistic input, such as reading comprehension or written expression. Deficits in working memory have been linked to impairments in social skills (McQuade, Murray-Close, Shoulberg, & Hoza, 2013), early numeracy skills (Toll & Van Luit, 2013), reasoning (Kail, 2007), problem solving (Passolunghi & Mammarella, 2012), reading (Wang & Gathercole, 2013), and attention (Awh & Jonides, 2001). Working memory is critical for academic, behavioral, and social functioning and requires the processing and manipulation of phonological and visual-spatial information.

1.3 Working Memory in Intellectual Disabilities

Individuals with intellectual impairment and developmental disabilities typically present with memory deficits. However, there are conflicting viewpoints regarding the relationship of working memory performance in populations with developmental disabilities in regards to how memory impairments develop over time. Swanson and Siegel (2001) provide a review of various issues that emerge when examining working memory profiles of individuals that have developmental disabilities. Two theories have emerged to help explain the cognitive processing of children with intellectual disabilities. The developmental model (Zigler, 1969) suggests that children with intellectual disabilities have cognitive profiles that are similar to that of typically developing children, only delayed in their development. In support of a developmental model, Henry and MacLean (2002) compared working memory performance in children with intellectual disabilities that were matched on mental and chronological age. Results indicated that children with intellectual disabilities performed at a similar level as the

control group that was matched on mental age suggesting that their working memory abilities were delayed and not the result of a specific deficit.

In contrast, the deficit model suggests that a specific deficit is responsible for impairment in cognitive processes regardless of mental capability. Conner et al. (2011) examined the memory profiles of individuals from three genetic syndromes associated with intellectual impairment. Distinct memory profiles emerged for each of the three etiologies (i.e. Down syndrome, Williams syndrome, and fragile X syndrome) providing evidence that the type and intensity of impairment is variable in each syndrome regardless of the global presenting intellectual disability. Individuals with Down syndrome had strengths in visual memory, but demonstrated poor verbal working memory. Williams syndrome was associated with relatively good visual and verbal working memory in contrast to individuals with fragile X syndrome who displayed severe impairments in both visual and verbal working memory. A recent study examined the cognitive profile of individuals with Down syndrome and found similar deficits in the working memory systems of the phonological loop and central executive which is consistent with other research examining the cognitive phenotype of working memory (Conner et al., 2011; Lanfranchi, Jerman, & Vianello, 2009).

Alloway et al. (2009) provided comparable evidence in her study examining whether the working memory skills of students with various developmental disorders presented with selective memory deficits associated with their diagnoses (Specific Language Impairment, Developmental Coordination Disorder, Attention-Deficit/Hyperactivity Disorder (ADHD), and Asperger Syndrome). Individuals that had impairments in their language displayed selective deficits in working memory and verbal

short-term memory. Also, children that had motor impairments (i.e. Developmental Coordination Disorder) had associated specific deficits in visuospatial short-term and working memory. Children with Asperger's syndrome displayed deficits only in their short-term memory, while children with ADHD presented with deficits in both domains of working memory (verbal and visuospatial). Although it is agreed that children with developmental disabilities present with working memory deficits, debates arise to whether impairments are a function of a unitary cognitive deficit or are a part of multi-faceted cognitive profile. These considerations further validate that memory is a complex cognitive process that involves multiple inter-related processes that are often associated, but also can be independently impacted.

1.4 Working Memory and FXS

In the past few years, there have been increased efforts to better define the cognitive phenotype associated with FXS (Baker et al., 2011; Hooper et al., 2008). Since FXS is a developmental disorder and the most common genetic condition responsible for intellectual disabilities (Crawford et al. 2002), past efforts have examined FXS in regard to measures of general intelligence (Hooper, Hatton, Baranek, Roberts, & Bailey, 2000). However, recently the focus has switched to study specific cognitive processes to better understand the cognitive phenotype associated with this unique population.

Despite recent attempts to identify a cognitive profile associated with FXS, little research has been conducted in the area of working memory. Although there is consensus that children with FXS present with impairments in working memory performance (Baker et al., 2011; Conners et al., 2011; Hooper et al., 2008; Lanfranchi,

Cornoldi, Drigo, & Vianello, 2009; Ornstein et al., 2008), there have been mixed results in regard to whether deficits in working memory are globally impaired or if they impact specific subsystems of working memory (i.e. visual-spatial processing, verbal/phonological processing, central executive, etc.). Past research has found that males with FXS perform lower on specific memory tasks that involve either visual-spatial processing (Ornstein et al., 2008; Schapiro et al., 1995) or verbal/phonological processing (Baker et al., 2011) then what would be expected at their developmental level. In contrast, some studies have found global working memory deficits in males with FXS on both verbal and visuospatial memory tasks (Munir et al., 2000; Ornstein et al., 2008) compared to typically developing controls after controlling for mental age. These findings led to work examining whether an overall deficit in working memory may be better explained by the attention, task complexity, or other individual differences that may have an impact on working memory task performance.

One study (Lanfranchi et al., 2009) assessed whether 15 boys with FXS differed from 15 typically developing controls after controlling for mental age on working memory tasks that differed in complexity on both verbal and visual-spatial domains. No significant differences were found in performance between the groups on tasks that had lower levels of complexity; however, as tasks became more complex disparities between the groups became apparent with boys with FXS performing worse than the typically developing controls. Similar results have been attained in tasks that analyze low vs. high levels of attentional processing in males with FXS (Cornish, Sudhalter, & Turk, 2004), which suggest that boys with FXS may have a specific deficit in the central executive domain of working memory (Scerif, Cornish, Wilding, Driver, & Karmiloff-Smith, 2004)

and have difficulty holding and processing information regardless of whether it is verbal or visual-spatial information. Cornish et al. (2009) also found a positive correlation between increased CGG repeat and greater impairment to the central executive component of working memory, which suggests genetic influences, may contribute to cognitive impairments found in individuals with FXS. Therefore, individual biological differences may account for some of the variability displayed in working memory performance.

1.5 Salivary Cortisol

The importance of identifying and examining various biomarkers to help explain the relationship of how physiological processes impact cognition and human development have been reported across multiple scientific disciplines (Tommasi, Peterson, & Nadel, 2009). The identification of specific biomarkers has provided an objective way to measure subjective constructs. This understanding of how biocognitive influences interact and impact an individual's development over time will increase the specificity of assessment and treatments.

Salivary cortisol has been frequently studied as a biomarker for psychological stress (Hellhammer, Wüst, & Kudielka, 2009). The hypothalamic-pituitary-adrenal (HPA) axis is a dynamic system that responds to and regulates physiological and behavioral reactions to stress. This complex system involves the secretion a corticotropin-releasing hormone, which signals the adrenal glands to release cortisol when an individual experiences stress (Jacobson, 2005). When an individual experiences an acute stressful event, the pattern and response to stress becomes adaptive in order to prepare the

individual to cope with the stressor (Dickerson & Kemeny, 2004). Therefore, acute stress can be reflected by the state of the individual and measured by the reactivity of cortisol after the event. However, when cortisol is chronically elevated by stress or disruptions in the regulatory processes, an individual's cognition and ability to learn may be impacted (Sapolsky, 2000; Wolf, 2003). Baseline levels of cortisol can act as a measure of chronic stress and resembles a trait-like characteristic of the individual.

1.6 Cortisol and Memory Performance

The relationship of stress on an individual's cognitive performance has been well documented in the literature (Smeets, Otgaar, Candel, & Wolf, 2008; Wolf, 2009), particularly the effects on memory performance (Oei, Everaerd, Elzinga, Van Well, & Bermond, 2006; Taverniers, Van Ruysseveldt, Smeets, & Von Grumbkow, 2010; Wolf, Schommer, Hellhammer, McEwen, & Kirschbaum, 2001). Although brief or acute stressful events can trigger the HPA axis to secrete cortisol in order to facilitate cognition as an adaptive mechanism, the opposing results can be seen when these mechanisms become saturated from chronic stress and cause disruptions in cognitive performance. Vedhara et al. (2000) examined short-term memory and found that increased levels of cortisol were associated with fewer words remembered in a word recall test. Experimental studies have also reported the effects of acute cortisol on working memory performance (Lupien, Gillin, & Hauger, 1999; Wolf et al., 2001) and have found that increased cortisol is associated with poorer working memory performance. These results suggest an inverse relationship between cortisol and working memory performance. However in individuals that have elevated levels of cortisol due to chronic stress or exaggerated reactivity to stress, these effects may be more pronounced.

The relationship of a naturalistic and maladaptive response to stress on memory and cognitive performance has been investigated. One study conducted by Mattarella-Micke and colleagues (2011) explored the relationship of individual differences in working memory capacity and math-anxiety. Math performance for individuals who had lower working memory capacities was not related to their cortisol level or math-anxiety. However, for individuals with higher working memory capacities and elevated cortisol, differences were seen dependent on their levels of math-anxiety with more anxious individuals performing worse than individuals with low anxiety. These results highlight the importance of including physiological measures to help explain potential cognitive mechanisms.

To our knowledge, no research has examined the relationship of cortisol and the Baddeley's domains of working memory (i.e. central executive, visual-spatial sketchpad, and phonological loop) or varying levels of working memory complexity. Also, the majority of studies examining the associations between working memory and cortisol have been conducted using adult samples of participants. Further investigation using varying measures of working memory and samples of children are needed to better explain the dynamic relationship of how physiological processes impact cognition especially during early development.

1.7 Cortisol and FXS

Early studies have examined how increased diurnal levels of cortisol correspond to the fragile X phenotype including increased behavior problems, social anxiety, withdrawal, and hyper-arousal (Hessl et al., 2002; Wisbeck et al., 2000). However, to

address whether levels of cortisol are related to state or trait-like characteristics of the individual, the effects of cortisol between discrete time points surrounding a task, such as baseline and reactivity, have been recently studied in regards to boys and girls with FXS. Hessl et al. (2006) found that increased cortisol reactivity to a social task resulted in more eye contact after controlling for baseline levels of cortisol. Results from these studies highlight the need to study multiple time points of cortisol surrounding a task to best account for whether the task elicited an acute stress reaction by measuring reactivity or if the effects are better explained by chronic stress measured by baseline cortisol levels.

Research has also examined how social behaviors in children with FXS are related to elevated salivary cortisol and increased autistic behaviors (Roberts et al., 2009), abnormal gaze patterns (Hessl et al., 2006) and more intense social escape behaviors (Hall , DeBernadis, & Reiss, 2006). However, to date, no research study has examined how cortisol is related to specific cognitive phenotypes associated with FXS. This highlights the need for further investigation regarding how the dynamic systems of biological, environmental, and cognitive factors interact and impact an individual's development and functioning.

1.8 Current Study

Working memory is a complex cognitive process that is involved in many higher order cognitive tasks involved with learning. It is important to study the development of working memory over time and the underlying physiological mechanisms that affect its development. By better understanding the interplay between multiple processes on cognition, more specified assessments and interventions can be implemented to target and

treat individuals over time. Salivary cortisol may explain some of the variance captured by individual differences that mediate working memory performance. Individuals with FXS are an ideal population to study because they are the result of a single gene disorder that has distinct physiological mechanisms that may impact certain cognitive outcomes.

The inconsistencies illustrated by recent literature provide evidence that this is an area that deserves more attention and investigation. Some of the discrepancies found in working memory performance in young males with FXS may be due to the limitations of working with small samples or cross sectional research designs. Consequently, no study has examined working memory performance over time using a longitudinal design in young boys with FXS. Additionally, to date, no study has looked at the relationship of how salivary cortisol is related to working memory over time in young boys with FXS or any developmental or intellectual disability. Therefore, the following research questions and hypotheses have been developed:

1. What is the relationship of working memory performance over time in boys with FXS compared to typically developing boys?

Hypothesis: Boys with FXS will have decreased working memory performance and have slower rates of growth over time compared to typically developing boys.

2. What is the relationship of salivary cortisol on memory performance over time in boys with FXS and typically developing boys?

Hypothesis: Boys with FXS will have increased measures of cortisol compared to typically developing boys. Increased measures of cortisol will be associated with reduced working memory performance.

CHAPTER 2

METHODS

2.1 Participants

Data were collected from a prospective longitudinal study of males with FXS at the University of North Carolina to examine patterns of memory, attention, and executive functioning over time in early development. Participants were recruited from a variety of sources including a national registry for FXS research, support groups, and advertising through schools and community centers near the University of North Carolina. In order to address each of the study's research questions, two datasets were created.

The first dataset includes a sample of 52 children with FXS and 52 typically developing (TD) children for a total of 104 participants to explain the relationship of memory performance over time in TD children and children with FXS. Demographic information provided for the 52 children with FXS indicated 81% (N=42) of the sample was identified as being Caucasian, while 19% (N=10) identified other racial/ethnic backgrounds. For the TD group, 85% (N=44) were Caucasian and 15% (N=8) identified other racial/ethnic backgrounds. Each participant was assessed 1-4 times with 12 months between each assessment. To control for mental age effects, the TD sample of children were matched to the FXS sample at the first time point of the longitudinal study by their mental age (FXS average mental age= 5.2 years; TD average mental age= 5.3). Demographic information is included in Table 2.1.

To answer the second research question of how measures of salivary cortisol are related to memory performance over time in children with FXS and TD children, a second dataset was created from the subset of participants from the first dataset who also had cortisol data. Salivary cortisol data were missing due to lack of participant compliance or errors in collecting the data. Also, assayed values indicating an error or contaminated sample were discarded. Measures of chronological age, mental age, auditory working memory, and memory for words for each group at each time point within the cortisol dataset were compared to the primary dataset using paired t-test analyses. No statistically significant differences ($p>.05$) were apparent between the two datasets indicating that the second subset of participants with cortisol data are representative of the larger primary dataset. The final cortisol sample includes 31 children with FXS and 49 TD children for a total of 80 participants and 154 data points. Each participant was assessed 1-3 times with 12 months between each assessment.

2.2 Measures

At each of the assessments, working memory, salivary cortisol, and mental age data were collected from both groups of participants. All data were collected during the same assessment period and the same order of assessment completion was adhered to.

2.2.1 Working Memory

Working memory scores were obtained through the administration of two subtests from the *Woodcock-Johnson Tests of Cognitive Abilities, Third Edition (WJ-III,* Woodcock, McGrew, & Mather, 2001). The two subtests on the WJ-III were used as separate measures of working memory instead of a single composite because of the

different cognitive demands required to complete the subtests and the differences that were seen across groups. To capture any potential discrepancies in performance between subtests and their relationships to measures of cortisol, the two separate subtests were used as measures of working memory in contrast to a single composite score of working memory.

The *Memory for Words* subtest is a measure of short-term memory that requires the participant to repeat a series of words that are unrelated in the exact order in which the items were presented orally. The participant begins with an item that is a single word and as the participant answers items correctly the span of words increases up to a series of seven words. The range of raw scores obtained through the *WJ-III* Memory for Words subtest is from 0 to 24. A participant receives a point for each word span sequence that is answered correctly. The subtest is discontinued after the participant answered three items in a section incorrectly. The median internal consistency reliability coefficient for the Memory for Words subtest is a .80. The present study used the W score as a measure of working memory for this subtest. The W score is a metric that uses an equal-interval scale that represents the same difference or amount of growth in a trait across measures, and also takes into account the difficult levels of all items of the measure (Jaffe, 2009). The W score is useful for reporting an individual's growth in a skill, ability or area of knowledge and is constructed to represent actual growth in the trait measured (Woodcock & Dahl, 1971). The W score was used in this longitudinal study as a stable metric of change and to protect against the floor effects reflected by standard scores Over the course of the 4 time periods of data collection, the FXS group had a total of 130 observations for the memory for words subtest (Time 1: N= 52, Time 2: N = 42,

Time 3: N= 24, Time 4: N= 11) and the TD group had a total of 111 observations for the memory for words subtest (Time 1: N= 52, Time 2: N= 40, Time 3: N=19). Table 2.2 provides descriptive data for the measure of Memory for Words performance used in this study.

The *Auditory Working Memory* subtest is a working memory measure that requires the participant to listen to words, that include both the names of numbers and objects in a mixed up order, and repeat the series of words back with the words of objects first and then the number words. The participant begins the task with an item that includes a word of a single object and a single number. As the participant answers items correctly, one point is awarded for the recitation of the correct sequence of objects and one point for the words of numbers. Therefore, the participant can obtain up to 2 points per item. As the items get more difficult, the span of words of objects and numbers increases up to a series of 4 number words and 4 object words. The Auditory Working Memory subtest is discontinued after the participant receives a score of 0 on three consecutive items. The median internal consistency reliability coefficient for the Auditory Working Memory subtest is a .80. Over the course of the 4 time periods of data collection, the FXS group had a total of 115 observations for the auditory working memory subtests (Time 1: N=44, Time 2: N= 37, Time 3: N= 23, Time 4: N= 10,) and the TD group had a total of 113 total observations for the auditory working memory subtest (Time 1: N= 52, Time 2: N=42, Time 3: N=19). The present study used the W score as a measure for working memory for this subtest. Table 2.2 provides descriptive data for the measure of Auditory Working Memory performance used in this study.

2.2.2 Salivary Cortisol

Samples of cortisol were collected from the participants at two time points during each assessment. The samples were acquired from the participants through the use of a salivette that was placed in the participant's mouth for 1-2 minutes. The initial sample that was collected occurred 15 minutes before the start of the assessment and is considered to be a measure of the participant's cortisol levels prior to the effects of testing. The first sample of salivary cortisol considered as the participant's "baseline" level of cortisol. The second sample of salivary cortisol that was collected from the participant was taken at the conclusion of the assessment and is included as a measure of that participant's reactivity during the assessment. The second sample of salivary cortisol is labeled as the "reactant" score. Additionally, the amount of change between cortisol levels at each sample was calculated by subtracting the baseline level of cortisol from the reactant level of cortisol. The amount of change in cortisol from each time sample functions as a measure of cortisol reactivity and provides a way to study the participants' physiological response to stress experienced from the assessment. Salivary cortisol was processed using the Salimetrics Salivary Cortisol Enzyme Immunoassay kit (EIA) and cortisol levels were collected using measures in micrograms/deciliters. Over the course of the 3 time periods of data collection, the FXS group had a total of 63 observations (Time 1: N=31, Time 2: N=19, Time 3: N=9). The TD group had a total of 91 observations (Time 1: N=49, Time 2: N=29, Time 3: N= 13). Table 2.3 provides descriptive data for the three measures of salivary cortisol in this study.

2.2.3 Mental Age

The Leiter-R (Roid & Miller, 1997) is measure of nonverbal intelligence. In order to obtain an overall IQ estimate, the *Brief IQ Screener* was used as a measure of each of the participant's overall cognitive functioning and as a covariate to working memory performance. The Brief IQ Screener on the Leiter-R provides a growth score, similar to the W score on the WJ-III, and was used to measure a participant's mental age (MA) in the present study. A growth score reflects growth of an individual's performance at a particular age, as well as towards the difficulty of items within the test battery. Typically developing children were matched to the FXS sample based on their MA at the first assessment. The Brief IQ Screener is comprised of four subtests and included Figure Ground, Form Completion, Sequential Order, and Repeated Patterns. The Leiter-R Brief IQ screener is suitable for individuals ages 2-20 and has consistent scores with other cognitive measures such as the Wechsler Intelligence Scale for Children-III (WISC; Wechsler, 1991). The internal consistency reliability coefficients of the Brief IQ screener range between .88 and .93 depending on the age of the individual. The growth score was used as a measure of the participants' mental age. A growth score was also obtained at each assessment for the two working memory subtests.

2.3 Procedure

The working memory, cognitive and salivary cortisol measures were completed within a larger neurocognitive battery of assessment. The study was initially described to parents that were interested in participating over a phone call or through a letter. Parents interested in having their children participate, and who met the inclusion/exclusion

criteria for the study, were invited for an initial assessment session. Informed consent and background information were obtained from both parents of typically developing children and children with FXS. Parents of typically developing children were invited to participate in an initial assessment where the child completed the Leiter-R Brief IQ Screener. Typically developing children who obtained results from the Leiter-R Brief IQ Screener in the average range and had a MA that was comparable to a participant with FXS were allowed to enroll in the study and complete the additional assessments.

Individual assessments were conducted primarily at the participants' home or school based on parental preference. A blocking procedure was used in the assessment battery and the order of the tests administered during each assessment was controlled for order effects. The assessment period ranged typically over a period of two days for each of the participants. Score calculations were double-checked at 100% and those data were double-entered and verified at 20% for accuracy.

2.4 Data Analysis

Multilevel modeling (MLM) was used to analyze a participant's working memory trajectories in SAS 9.2 PROC MIXED (SAS Institute, 2008). MLM is a powerful method of conceptualizing how an individual changes over time by allowing the analyses of both within and between-subject variance (Singer, 1998). MLM was used to analyze data from the present study because participants were assessed at multiple time points and because there was variation in the number of observations across participants.

MLM is composed of two hierarchical models that enables the simultaneous analysis of how each individual changes over time (Level 1 model) and how these

changes vary across subjects (Level 2 model) (Bryk & Raudenbush, 1987; Rogosa & Willett, 1985). The Level-1 portion of the MLM, which is also referred to as the individual growth model, represents the expected amount of change each individual in the population will endure during the time period under the study. The Level-2 component of the MLM represents the relationship between trajectories of change and time-invariant characteristics between individuals.

Table 2.1

Descriptive statistics for chronological and mental Age

	FXS		TD	
	M	SD	M	SD
Age at first assessment	121.26	19.82	61.42	10.37
Age across all assessments	132.99	21.54	69.4	13.68
Mental age at first assessment	62.29	8.58	64.04	10.08
Mental age across all assessments	64.58	8.32	74.68	17.44

Note. FXS= Fragile X Syndrome. TD= Typically Developing. Age and mental age are measured in months.

Table 2.2

Means and standard deviations on the subtests of memory for words and auditory working memory as a function of group and time

	Memory for Words			Auditory Working Memory		
Group/Time	N	M	SD	N	M	SD
FXS Time 1	52	419.94	20.57	44	449.84	14.35
FXS Time 2	42	424.17	22.40	37	451.97	14.77
FXS Time 3	24	417.13	21.33	23	457.30	18.73
FXS Time 4	11	432.45	26.25	10	467.20	20.67
TD Time 1	52	466.58	21.38	52	466.48	18.52
TD Time 2	40	483.18	17.96	42	482.00	17.63
TD Time 3	19	487.79	23.29	19	495.21	13.88

Note. FXS= Fragile X Syndrome. TD= Typically Developing. "Time" refers to sessions in which memory for words and auditory working memory assessments were administered.

Table 2.3

Means and standard deviations on measures of cortisol as a function of group and time

	Baseline Cortisol			Reactant Cortisol			Cortisol Change		
Group/Time	N	M	SD	N	M	SD	N	M	SD
FXS Time 1	31	.28	.39	27	.18	.09	27	-0.01	0.11
FXS Time 2	19	.34	.34	18	.24	.36	18	-0.10	0.21
FXS Time 3	9	.42	.79	9	.23	.25	9	-0.19	0.55
TD Time 1	49	.23	.22	46	.17	.24	46	-0.08	0.28
TD Time 2	29	.24	.58	27	.37	.66	27	-0.05	0.26
TD Time 3	13	.18	.11	13	.12	.06	13	-0.06	0.10

Note. FXS= Fragile X Syndrome. TD= Typically Developing. "Time" refers to sessions in which memory for words and auditory working memory assessments were administered.

CHAPTER 3

RESULTS

3.1 Preliminary Analyses for Working Memory

Preliminary exploratory analyses of the data were run to confirm the assumptions of hierarchical linear modeling and to aid in selecting variables and interactions to include into the model. To examine the assumption of normality, the residuals were analyzed using Q-Q plots. The patterns of the residuals and error terms suggested that the data was not being impacted significantly by outliers. Therefore, no data points were removed from the dataset. In order to test the assumption that the data is best represented by general linear model, the data were examined using empirical growth plots. To test the assumption of homoscedasticity of variance, standardized plots indicate that the variances of the residuals are equal across ages.

As shown by the "Spaghetti" plot in Figure 3.1 and Figure 3.2, the overall trends for both groups appear to be linear against age and the typical group has a higher overall average performance on both the memory for words and auditory working memory subtests. If we combine two plots together, the Figure 3.3 and Figure 3.4 display that two groups are distinctive and TD sample's performance on the memory subtests increased much faster against age than the FXS sample.

3.2 Primary Analyses for Working Memory

To address how working memory performance changes over time between FXS and the TD groups, two separate models were created to examine the fixed effects of age and group on the performance of each of the working memory subtests. Mental age was used as a covariate in each of the models. The variable for time was coded for months and represented the participant's chronological age. Chronological age was centered at the grand mean at the initial assessment, so that the parameters of the intercept can be interpreted as the average across groups. The Level-1 model that was evaluated was the unconditional means and growth model, which estimated the level and change in working memory performance over time across all the participants in the study.

Level-1*:* $Y_{ij} = ß_{0j} + ß_{1j}(AGE_{ij}) + e_{ij}$

Where:

Y_{ij}= the dependent variable (i.e. working memory performance) of the observation I for individual j.

$ß_{0j}$ = represents the true change intercept for individual j.

$ß_{1j}$ = represents the true change slope for individual j.

e_{ij} = the random error in the predictions of the unconditional model.

In order to test the hypothesis that working memory performance outcomes would be predicted by group over time controlling for mental age, a separate conditional model was created to test the effect of each outcome on the intercept and slope values in the

previous unconditional model. These Level-2 submodels allow for the analyses of systematic interindividual differences in change across participants with FXS and TD.

Level-2: $ß_{0j} = \gamma_{00} + \gamma_{01}GROUP + \gamma_{02}MENTAL + \zeta_{0i}$

$ß_{1j} = \gamma_{10} + \gamma_{11}GROUP + \gamma_{12}MENTAL + \zeta_{1i}$

Where:

γ_{00} = Population average true initial statues for TD children

γ_{01} = Difference in population average true initial status between TD children and FXS children controlling for mental age

γ_{02}= Differential of initial status of working memory performance for a one unit difference in cortisol controlling for the effect of group

γ_{10} = Population average rate of true change for TD children controlling for mental age

γ_{11} = Difference in population rate of true change between TD children and FXS children

γ_{12} = Differential of true change of working memory performance for a one unit difference in cortisol controlling for the effect of group

ζ_{0i} = Population residual variance of true initial status, controlling for group

ζ_{1i} = Population residual variance of true rate of change, controlling for group

Scatter plots of each of the working memory subtests versus mental age were created to address adding mental age as a covariate into the model. The scatter plots reveal that

working memory performance increases linearly against mental age. As expected in Figure 3.5 there was some colinearity between chronological age and mental age, since as a child ages their mental age usually increases. Based on these observations, the following variables and interactions were included into the final linear mixed effect model:

$$Y_{ij} = \beta_0 + \beta_1 Group_i + \beta_2 Mental_i + \beta_3 Age_{ij} + \beta_4 Group_i \times Age_{ij} + \beta_6 Mental_i \times Age_{ij} + b_{0i} + b_{1i} Age_{ij} + \epsilon_{ij}$$

3.3 Unconditional Model for Working Memory

In order to evaluate variation in memory performance, two models were evaluated to test the null hypotheses that memory performance have similar levels and variation in change over time across all the participants. The first model that was fit was the unconditional means model which described the level of outcome variation of memory performance across all of the participants in the absence of the predictors of chronological age and group. Results of the unconditional model displayed indicate that there is significant variability of mean levels of working memory performance across participants in both auditory working memory and memory for words ($p<.01$) .

Unconditional Means Model: $Yij = \gamma 00 + \zeta 0i + \varepsilon ij$

Where:

$\gamma 00$= Grand mean across individuals and occasions

$\zeta 0i$= Person-specific means

ε_{ij} = Within-person deviations

The second model is the unconditional growth model and introduced the predictor of chronological age into the Level-1 submodel. This model described variation in the trajectory of working memory performance over time across all participants. In both the subtests, memory for words and auditory working memory, there was significant variability in the participants' true change trajectory ($p<.01$). These results indicate that the outcome variability of working memory performance may be better explained by additional predictors.

Unconditional Growth Model: $Yij = \gamma_{00} + \gamma_{10} TIMEij + [\zeta 0i + \zeta 1i TIMEij + \varepsilon ij]$

Where:

γ_{00} = Average true initial status at the average age across groups

γ_{10} = Average true rate of change

$[\zeta_{0i} + \zeta 1i TIMEij + \varepsilon_{ij}]$ = Composite residual

3.4 Conditional Model for Working Memory

In order to test our hypothesis that working memory performance would be predicted by group over time, we fit a final model to include group (FXS or TD) as a predictor of both initial status and change with mental age serving as a covariate for both subtests of working memory. Table 3.1 and Table 3.2 provide the results of the final model. Controlling for the effects of mental age, significant fixed effects for group was related to performance on both the memory for words (B=57.53 (7.47), $p<.001$) and auditory working memory subtests (B=31.43 (5.55), $p<.001$). The estimated differential

in memory for words performance between children with FXS and TD is 57.53 ($p<.05$) and 31.43 for the auditory working memory subtest, with the TD performing higher than the FXS group on both subtests. However, despite these differences between the two groups, results of the rate of change in working memory performance over time indicate that there are no significant differences after controlling for mental age on either of the subtests.

The proportion of variance, pseudo R^2 values, explained by the models were calculated using methods provided by Singer and Willet (2003) listed below. In both subtests the variance components of within-and between-subjects indicate that there may be some potentially explainable residual variance that can account for the variability in the sample which justifies the addition of other predictors into the model, such as measures of cortisol.

$$\text{Pseudo } R^2 = \frac{\text{variance unconditional model} - \text{variance conditional model}}{\text{variance unconditional model}}$$

3.5 Preliminary Analyses for Cortisol

To address the unexplained variance indicated by the residuals in the previous model, measures of salivary cortisol were added into the model. The second research question included the addition of the three measures of salivary cortisol (baseline, reactant, and change) to determine the relationship of how cortisol, which is impacted by acute and chronic stress, influences working memory over time between the two groups (FXS and TD). Thus the growth model created to answer the first research question was used a foundation for the addition of the three cortisol measures. Each of the three

cortisol measures were added into the model above separately for a total of six models, three models for each outcome of working memory performance.

Preliminary exploratory analyses of the data were run to confirm the assumptions of hierarchical linear modeling and to aid in selecting variables and interactions to include into the model. Pearson correlations were conducted to determine if the participants' cortisol levels (baseline, reactant, and change), mental age, and chronological age were correlated with the working memory performance of the memory for words and auditory working memory subtests. Table 3.3 and Table 3.4 further describe the results of the Pearson correlations for each group. To examine the assumption of normality, the residuals were analyzed using Q-Q plots. The patterns of the residuals and error terms suggested that the data was not being impacted significantly by outliers. However, since all three measures of cortisol (baseline, reactivity, and change) violated the assumption of normality a log transformation was performed on the each of the cortisol levels obtained from the EIA and was used as a measure of salivary cortisol. Therefore, no data points were removed from the dataset. In order to test the assumption that the data is best represented by general linear model, the data were examined using empirical growth plots. Standardized plots indicate that the variances of the residuals are equal across ages which satisfy the assumption of homoscedasticity of variance.

3.6 Primary Analyses for Cortisol

In order to answer the second research question, measures of cortisol were added into the final model addressed in the first research question above. The model was fit to

examine the variability in memory performance in the two subtests with cortisol, group, and chronological age serving as predictors while controlling for effects of mental age. The three measures of cortisol were modeled independently for each of the two subtests of working memory, memory for words and auditory working memory, for a total of six separate final models.

Using the same unconditional and conditional models explained previously for the first research question, results indicate that the cortisol data have significant variability in the level and change trajectories over time. Therefore both null hypotheses for the unconditional means and unconditional growth model were rejected ($p<.05$) indicating that the variability in working memory performance may be better explained by additional predictors.

3.7 Conditional Model for Cortisol

3.7.1 Memory for Words

The first model includes group (FXS and TD) and baseline cortisol (cort_B) as predictors of working memory performance over time on the memory for words subtest. Controlling for the effects of mental age on the performance of the memory for words subtest, significant working memory outcomes were related to differences in group and baseline cortisol over time (B= 0.48 (.19), $p <.05$). Boys in the TD group performed better on the memory for words subtest (B=54.22 (9.24) $p<.05$), had lower measures of baseline cortisol (B=1.39 (.403), $p<.05$), and displayed greater rates of growth in memory scores (B=1.39 (.403), $p<.05$) compared to boys with FXS. The results of the effects of

Model 1 are presented in Table 3.5. Figure 3.6 also shows the relationship of baseline cortisol between each of the groups at each time point.

The second model includes group (FXS and TD) and reactant cortisol (cort_R) as predictors of working memory performance over time on the memory for words subtest. Controlling for the effects of mental age on the performance of the memory for words subtest, working memory outcomes were not related to group or baseline cortisol changes over time (p's >.05). Although boys in the TD group performed better on the memory for words subtest (B=53.62 (9.66) p<.05) there were no significant differences in reactant cortisol (p>.05) or the change in memory for words performance over time (p>.05) compared to boys with FXS. The results of the effects of reactant cortisol on memory for words performance are presented in Table 3.6.

The third model includes group (FXS and TD) and the change in cortisol between baseline and reactivity (cort_C) as predictors of working memory performance over time on the memory for words subtest. Controlling for the effects of mental age on the performance of the memory for words subtest, working memory outcomes did not reach statistical significance in relation to differences in group or cortisol change over time (p's >.05). However, trends in working memory outcomes over time in group (p=.0851) and cortisol change (p=0681) are approaching a relationship with the performance on the memory for words subtest. Similarly to the other two models of memory for words performance, boys in the TD group performed better on the memory for words subtest (B=54.22 (9.24) p<.05) compared to boys with FXS. The results of these effects are presented in Table 3.7.

3.7.2 Auditory Working Memory

The fourth model includes group (FXS and TD) and baseline cortisol (cort_B) as predictors of working memory performance over time on the auditory working memory subtest. Controlling for the effects of mental age on the performance of the auditory working memory subtest, working memory outcomes did not relate to differences in group or baseline cortisol over time (p's >.05). Working memory outcomes were only related to group, in that boys in the TD group performed better on auditory working memory subtest (B=27.71 (6.81) p<.05) compared to boys with FXS. There was no significant difference in baseline cortisol (p>.05) between groups. The results of these effects are presented in Table 3.8.

The fifth model includes group (FXS and TD) and reactant cortisol (cort_R) as predictors of working memory performance over time on the auditory working memory subtest. Controlling for the effects of mental age on the performance of the auditory working memory subtest, working memory outcomes did not relate to group or reactant cortisol over time (p's >.05). Working memory outcomes were only related to group in that boys in the TD group performed better on auditory working memory subtest (B=28.02 (7.07) p<.05) compared to boys with FXS. There was no significant difference in reactant cortisol (p>.05) between groups. The results of the effects of reactant cortisol on auditory working memory performance are presented in Table 3.9.

The sixth model includes group (FXS and TD) and cortisol change (cort_C) as predictors of working memory performance over time on the auditory working memory subtest. Controlling for the effects of mental age on the performance of the auditory

working memory subtest, working memory outcomes did not relate to group or cortisol change over time (p's >.05). Group predicted the only significant relationship to working memory performance in that boys in the TD group performed better on auditory working memory subtest (B=28.17 (6.88) p<.05) compared to boys with FXS. Change in cortisol was not related to working memory performance (p>.05). The results of these effects are presented in Table 3.10.

Table 3.1

Results of MLM on Memory for Words

Parameter	Estimate	Std. Error	df	t	Sig.
Intercept	0.19	80.88	101	0	0.998
Group	57.53	7.47	47	7.7	.0001*
Age	-2.17	2.16	84	1.01	0.318
Mental Age	0.78	0.19	47	4.19	.0001*
Group*Age	0.32	0.21	47	1.51	0.137
Age*Mental Age	0.003	0.004	47	0.9	0.373

Note. Age= chronological age. Age and mental age were reported in months. * $p < .05$.

Table 3.2

Results of MLM on Auditory Working Memory

Parameter	Estimate	Std. Error	df	t	Sig.
Intercept	-18.05	62.55	101	0.29	0.773
Group	31.43	5.55	40	5.66	<.0001*
Age	-1.11	1.74	78	0.64	0.527
Mental Age	0.93	0.14	40	6.5	<.0001*
Group*Age	0.25	0.16	40	1.59	0.119
Age*Mental Age	0.002	0.003	40	0.57	0.571

Note. Age= chronological age. Age and mental age were measured in months. * $p < .05$.

Table 3.3

Pearson Correlations among Predictors and Outcome Variables for the Typically Developing Group

	1	AWM	MFW	Mental Age	Baseline Cortisol	Reactivity Cortisol	Cortisol Change
Age	--	.71	.55	.86	.05	.07	.06
AWM		--	.61	.74	.18	.06	-.15
MFW			--	.53	.27	.27	.03
Mental Age				--	.06	.06	.04
Baseline Cortisol					--	.80	-.20
Reactivity Cortisol						--	.43
Cortisol Change							--

Note. AWM = Auditory Working Memory. MFW = Memory for Words.

Table 3.4

Pearson Correlations among Predictors and Outcome Variables for FXS group

	1	AWM	MFW	Mental Age	Baseline Cortisol	Reactivity Cortisol	Cortisol Change
Age	--	.14	-.02	.3	.01	.08	.05
AWM		--	.53	52	-.15	-.01	.06
MFW			--	.55	-.18	.04	.29
Mental Age				--	-.42	.22	.46
Baseline Cortisol					--	.73	-.80
Reactivity Cortisol						--	-.16
Cortisol Change							--

Note. AWM = Auditory Working Memory. MFW = Memory for Words.

Table 3.5

Results of MLM on Baseline Cortisol and Memory for Words

Parameter	Estimate	Std. Error	df	t	Sig.
Intercept	144.57	103.29	76	1.4	0.166
group	54.22	9.24	18	5.87	<.0001*
age	-1.77	0.54	48	3.25	0.002*
mental age	0.52	0.23	18	2.25	0.037*
cort_B	9.45	3.05	18	3.1	0.006*
group*age	1.39	0.40	18	3.46	0.002*
age*cort_B	-0.66	0.27	18	2.49	0.023*
group*age*cort_B	0.48	0.19	18	2.45	0.025*

Note. Age= chronological age. Cort_B= baseline cortisol. Age and mental age were measured in months. * $p < .05$.

Table 3.6

Results of MLM on Reactant Cortisol and Memory for Words

Parameter	Estimate	Std. Error	df	t	Sig.
Intercept	95.4266	109.6	72	0.87	0.3868
group	53.6284	9.6618	16	5.55	<.0001*
age	-0.2687	0.7007	44	0.38	0.7032
mental age	0.6075	0.248	16	2.45	0.0262*
cort_R	3.0357	3.4193	16	0.89	0.3878
group*age	0.2375	0.5232	16	0.45	0.656
age*cort_R	0.1307	0.2845	16	0.46	0.6522
group*age*cort_R	-0.1217	0.2127	16	0.57	0.5752

Note. Age= chronological age. Cort_R= reactant cortisol. Age and mental age were measured in months. * $p < .05$.

Table 3.7

Results of MLM on Cortisol Change and Memory for Words

Parameter	Estimate	Std. Error	df	t	Sig.
Intercept	114.41	111.34	72	1.03	0.3076
group	54.5769	9.943	16	5.49	<.0001*
age	-0.5118	0.3566	44	1.44	0.1583
mental age	0.5508	0.2517	16	2.19	0.0439*
cort_C	21.1382	12.2616	16	1.72	0.104
group*age	0.5034	0.2742	16	1.84	0.0851
age*cort_C	2.3149	1.1833	16	1.96	0.0681
group*age*cort_C	-1.5568	0.7989	16	1.95	0.0691

Note. Age= chronological age. Cort_C= cortisol change. Age and mental age were measured in months. * $p < .05$.

Table 3.8

Results of MLM on Baseline Cortisol and Auditory Working Memory

Parameter	Estimate	Std. Error	df	t	Sig.
Intercept	33.5695	79.6567	76	0.42	0.6746
group	27.7083	6.8106	17	4.07	0.0008*
age	-0.2387	0.4386	46	0.54	0.5889
mental age	0.8386	0.1804	17	4.65	0.0002*
cort_B	1.3388	2.5097	17	0.53	0.6006
group*age	0.2312	0.3262	17	0.71	0.488
age*cort_B	0.01721	0.2224	17	0.08	0.9392
group*age*cort_B	0.05113	0.1649	17	0.31	0.7602

Note. Age= chronological age. Cort_B= baseline cortisol. Age and mental age were measured in months. * $p < .05$.

Table 3.9

Results of MLM on Reactant Cortisol and Auditory Working Memory

Parameter	Estimate	Std. Error	df	t	Sig.
Intercept	24.0633	81.4744	72	0.3	0.7686
group	28.0181	7.0663	16	3.97	0.0011*
age	-0.8061	0.538	42	-1.5	0.1415
mental age	0.8634	0.1846	16	4.68	0.0003*
cort_R	2.8235	2.617	16	1.08	0.2966
group*age	0.7332	0.4078	16	1.8	0.0911
age*cort_R	-0.2709	0.2218	16	1.22	0.2397
group*age*cort_R	0.2024	0.1685	16	1.2	0.247

Note. Age= chronological age. Cort_R= reactant cortisol. Age and mental age were measured in months. * $p < .05$.

Table 3.10

Results of MLM on Cortisol Change and Auditory Working Memory

Parameter	Estimate	Std. Error	df	t	Sig.
Intercept	3.6359	81.0692	72	0.04	0.9644
group	28.1677	6.8849	16	4.09	0.0009*
age	-0.2456	0.2539	42	0.97	0.3389
mental Age	0.8925	0.1828	16	4.88	0.0002*
cort_C	-7.0631	9.5894	16	0.74	0.4721
group*age	0.3166	0.1979	16	1.6	0.1291
age*cort_C	-0.3555	0.9103	16	0.39	0.7013
group*age*cort_C	0.2919	0.6223	16	0.47	0.6453

Note. Age= chronological age. Cort_C= cortisol change. Age and mental age were measured in months. * $p < .05$.

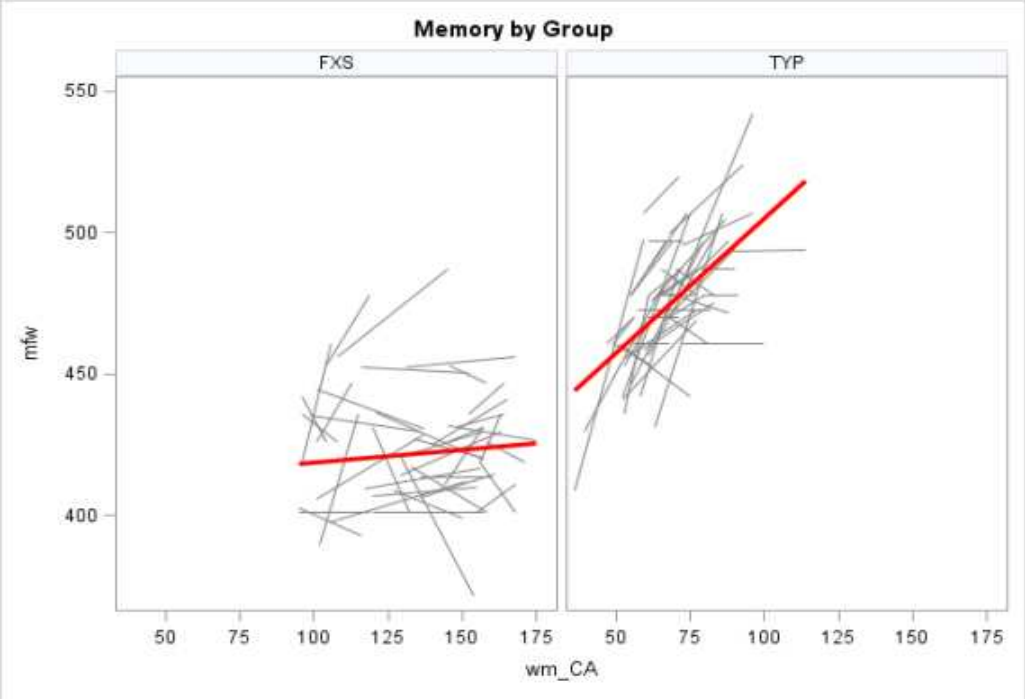

Figure 3.1 Spaghetti plots showing linear trends of performance on the memory for words subtest across FXS and TD groups plotted against chornological age.

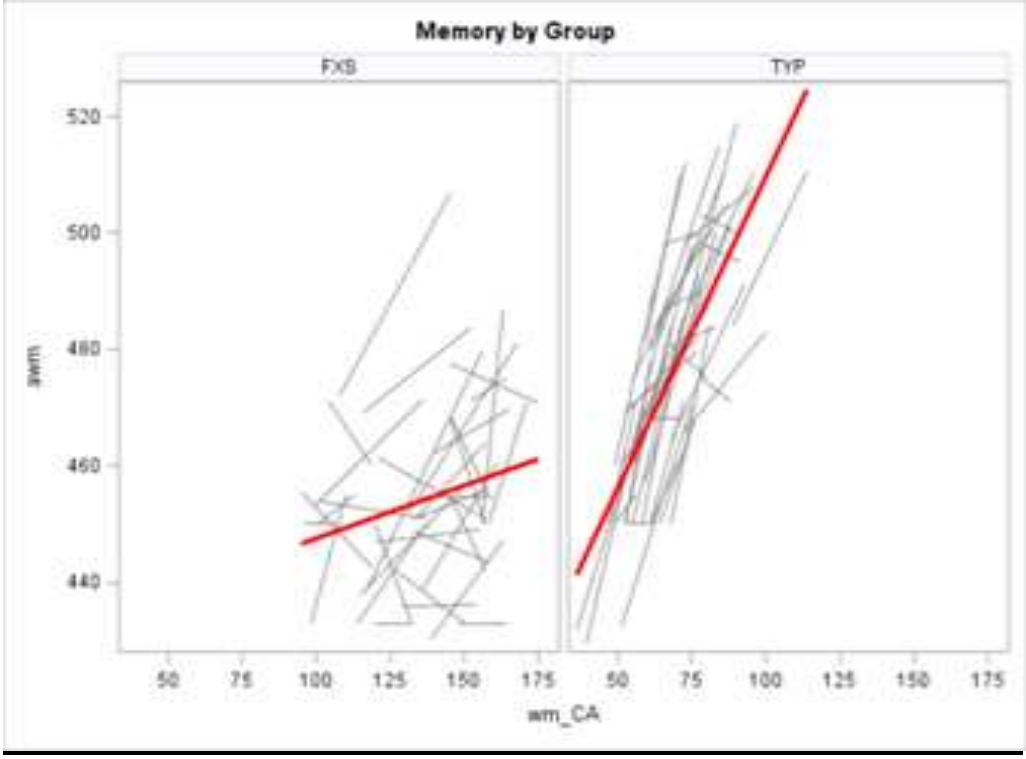

Figure 3.2 Spaghetti plots showing linear trends of performance on the auditory working memory subtest across FXS and TD groups plotted against chornological age.

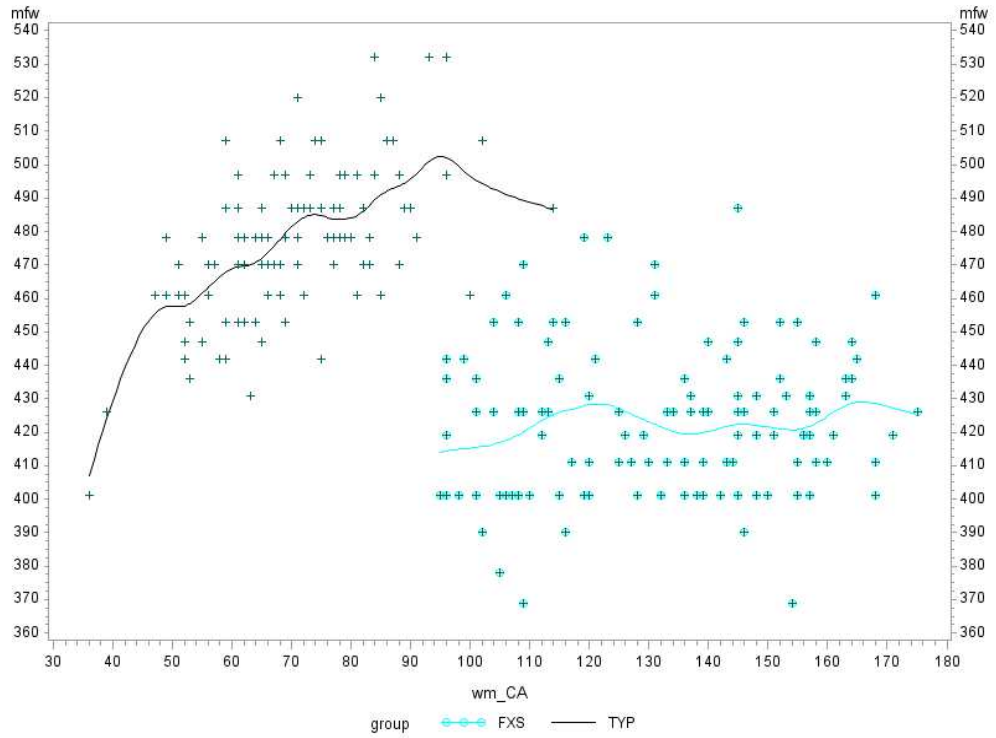

Figure 3.3 Combined plots of memory for words performance for both groups across chronological age.

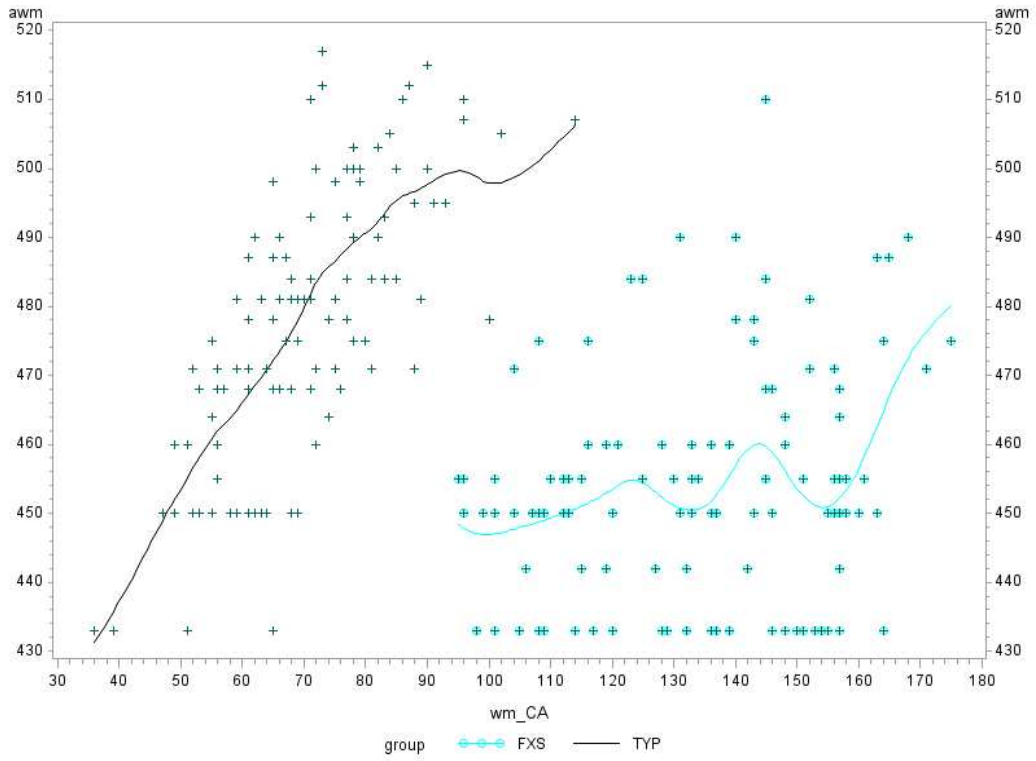

Figure 3.4 Combined plots of auditory working memory performance for both groups across chronological age.

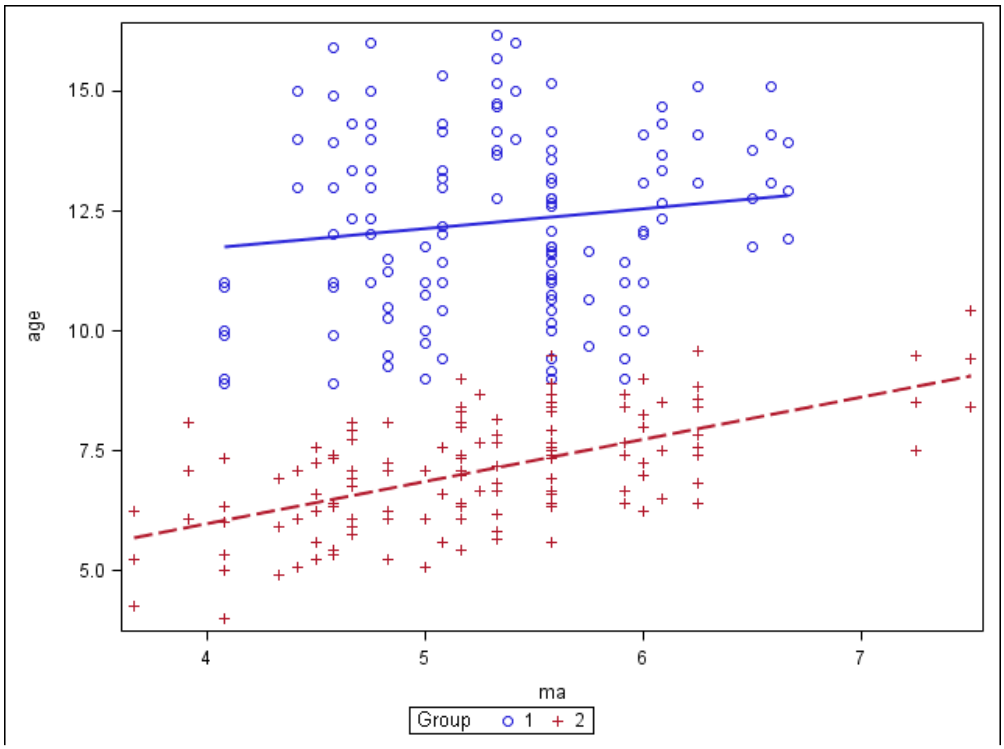

Figure 3.5 Scatterplot of mental age against chronological age for each groups.

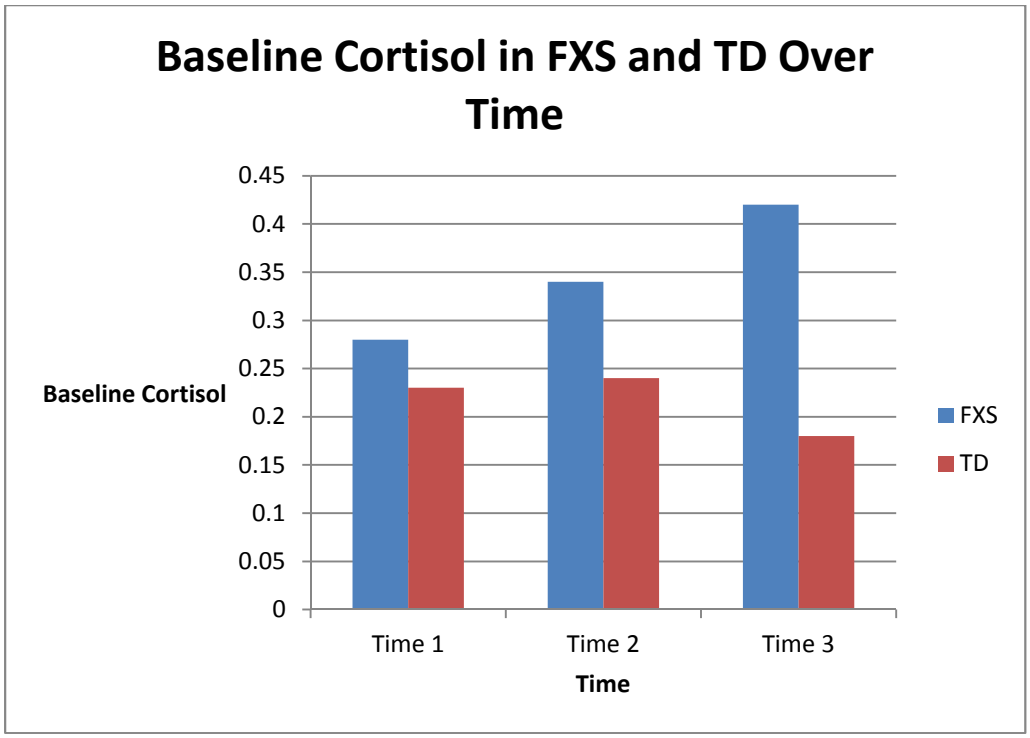

Figure 3.6 Bar graph of baseline cortisol at each time point for FXS and TD groups.

CHAPTER 4

CONCLUSION

Working memory is a complex cognitive construct involved in many aspects of an individual's behavioral, academic, and social functioning. The aims of the current study were to gain a better understanding of how working memory develops over time in boys with FXS compared to typically developing mental age matched boys. Additionally, we wanted to examine how physiological mechanisms, such as salivary cortisol, influence working memory development in boys with FXS compared to typically developing mental age matched boys. To investigate the relationships of how developmental trajectories of group (FXS or TD) and measures of cortisol (baseline, reactivity, and change) influence working memory performance, we utilized multilevel modeling to answer our research questions. Our results suggest that after controlling for mental age, boys with FXS performed worse on both measures of working memory (i.e. memory for words and auditory working memory) compared to boys who were typically developing. However, despite these differences in working memory performance between both groups, no significant differences between the groups were found when the rate of growth was analyzed in working memory performance of both subtests over time.

When we investigated the relationships of salivary cortisol and memory performance between the two groups, our findings indicate that boys with FXS who had higher measures of baseline cortisol displayed slower rates of growth in performance on

the memory for words subtest. However, these relationships of baseline cortisol and working memory performance were not evident on performance on the auditory working memory subtest. Also, there were no significant differences between groups on reactant cortisol or cortisol change for either of the subtests of working memory performance. Our results highlight the complexity of the relationship between cognition and physiological mechanisms and warrant the need to further examine dynamic factors that are related to the cognitive deficiencies seen in FXS.

4.1 Working Memory

Our results are consistent with past literature that found boys with FXS have decreased working memory performance compared to typically developing boys (Baker et al., 2011; Hooper et al., 2008; Munir et al., 2000). These findings are congruent with theory in support of a deficit model of cognitive impairment in groups with impaired intellectual functioning since disparities were seen between the groups despite being matched on mental age (Conner et al., 2011). Therefore, specific cognitive deficits in working memory best explain why boys with FXS performed worse on both measures of working memory compared to boys who are typically developing and matched on mental age.

However our findings did not support our hypotheses that boys with FXS will also have slower rates of growth of memory performance over time compared to boys that are typically developing. We did not find any significant differences in the developmental trajectories of working memory performance over time between the groups in either of the working memory subtests after controlling for mental age. This

finding suggests that, although boys with FXS overall perform worse on measures of working memory compared to typically developing boys, the rates of growth in working memory performance over time are not significantly different after controlling for mental age. These results are novel findings, since no study to our knowledge has examined the relationship of working memory performance in boys with FXS over time in a longitudinal design.

One reason for these unexpected findings may be accounted for by the differences in chronological age between boys with FXS compared to the typically developing boys who were matched on mental age. Although each of the groups had similar mental ages at the first assessment, the FXS group had a higher average chronological age (M= 11.1 years, range= 7-12 years) across all assessments compared to the typical group's chronological age (M= 5.8 years, range= 2-7years). Past research has shown that working memory capacity increases linearly with maturation (Alloway et al., 2006). Thus, the younger typical group may have a reduced working memory capacity as a function of their younger chronological age compared to the older FXS group. Although we did find significant group differences in working memory performance, the amount of growth over time may be limited by the younger TD group's working memory capacity until they reach a certain chronological age. This may explain why we did not find significant differences in growth or change in working memory performance between the groups after controlling for mental age.

4.2 Cortisol

Our results are consistent with previous research suggesting that individuals with FXS have heightened levels of cortisol (Hessl, Rivera, & Reiss, 2004; Roberts et al., 2009), specifically in measures of baseline cortisol (Hessle et al., 2006). In support of our hypothesis, our data indicate that boys with FXS have elevated baseline cortisol that is associated with lower performance on the memory for words subtest of working memory compared to typically developing mental age matched boys which supports past literature of memory performance and physiological measures of stress (Mattarella-Micke et al., 2011; Wolf et al, 2001). Although we found relationships between baseline levels of cortisol and working memory performance on the memory for words subtest, it appears to be specific to only the memory for words subtest.

One implication for these findings may be associated with the increased cognitive complexity of the auditory working memory subtest compared to the memory for words subtest. The auditory working memory subtest required the participants reorder a series objects and numbers, whereas the memory for words subtest required the participants to repeat back only a series of words. Although we used W scores to analyze our data, our participant's raw scores in suggest potential floor effects across both groups. Therefore, there may have not been enough variability in performance on the more cognitive demanding subtest of auditory working memory to detect a relationship of working memory performance and cortisol.

Additionally, we failed to find relationships between working memory performance on both subtests and measures of reactant cortisol and cortisol change in

boys with FXS and typically developing mentally age matched boys. One potential reason explaining why our results failed to support our hypothesis in regards to cortisol reactivity and change in our FXS group can be accounted for their elevated baseline cortisol levels. Boys with FXS had increased levels of baseline salivary cortisol indicating before testing took place. Therefore, higher baseline cortisol could impact how much boys with FXS could react to the stress of testing given that they were already experiencing a heightened level of stress. Consequently, this also impacts the amount of cortisol change that could occur between baseline and reactant cortisol measures and reduces the variability seen between measures.

In summary, our results suggest a specific relationship between of baseline cortisol and working memory performance on the memory for words subtest in FXS. Implications for these findings are well documented in the literature and suggest that increased levels of baseline cortisol reflect a measure of chronic stress which can negatively impact learning and memory performance (Oei et al., 2006; Taverniers et al., 2010; Wolf, 2009). This consistency reflects that physiological features associated with FXS are also linked with other cognitive outcomes (Taverniers et al., 2010; Wolf et al, 2001). While no research has investigated the relationships between working memory performance and salivary cortisol in FXS or other populations with intellectual or developmental impairments, our results suggest that this group and potentially others are sensitive to the effects of stress and working memory performance.

4.3 Limitations/Future Directions

Our preliminary findings linked physiological measures of baseline cortisol to working memory performance in FXS and used strong methodology to answer our research questions. Our study utilized a longitudinal design, mental age matched TD controls, and multiple measures of working memory and cortisol. However, although we matched our typically developing group of boys to the FXS group on mental age at the first time point, we did not control for the developmental effects of chronological age. Since our two groups belong to different chronological age groups, developmental factors associated with maturation may influence cognitive components of working memory capacity, as well as biological mechanisms. Also when creating a subset of participants who had cortisol data from our first dataset, we encountered missing cortisol data from some of the participants, Therefore, due to experimental error during collection or deficient quantities, our cortisol data is limited compared to our working memory dataset. Although our preliminary analyses did not detect significant differences between each dataset in regards to age, group, mental age, and working memory performance, the reduced number of participants may influence the amount of power to detect effects.

Future research should address the inclusion of mental age and chronological age matched controls, as well as the addition of another comparison group with intellectual deficits in a longitudinal design. Also, to examine how dynamic factors interact and impact development over time, environmental (ex. maternal factors, family dynamics, etc.), behavioral (ex. arousal, attention, mental health symptomology), and genetic (ex. CGG repeats) factors should be included in prospective longitudinal analyses. Future studies may want to consider adding supplementary physiological measures, such as

vagal tone or heart rate, to study how physiological arousal impacts cognition and behavior in populations with intellectual and developmental vulnerabilities (Roberts et al., 2001; Hall, Lifhtbody, Huffman, Lazzeroni, & Reiss, 2009). Additionally, although the current study used phonological measures of working memory to address the relationships of cortisol and working memory performance over time, potential studies may want to explore how working memory tasks that include both visual-spatial and phonological properties develop and change over time. Also, studying working memory tasks with varying complexities may further provide answers to distinguishing a cognitive phenotype in FXS.

The findings of this current study represent preliminary examinations of investigating the development of working memory over time in boys with FXS and typically developing mental age matched boys. To better understand how physiological factors influence working memory development, this study also examined the relationship of cortisol and working memory performance over time. Understanding how physiological factors impact working memory performance is important, given that working memory is a complex cognitive process that influences an individual's academic, behavioral, and social functioning. Furthermore, it is critical to study how dynamic factors develop and impact cognition in both atypical and typically developing populations over time. Our work also offers a basis for future studies to explore how theoretical models of the etiology of intellectual impairment best describes individuals with FXS in order to inform interventions specific to their unique cognitive and biological profiles.

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